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*Original scientific paper*

# RELATIONSHIP BETWEEN GENETIC DISTANCE, SPECIFIC COMBINING ABILITIES AND HETEROSIS IN MAIZE (*Zea mays* L.)

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Six maize inbred lines for which we assumed that have a similar genetic background were selected for the study. Inbred lines were crossed according to incomplete diallel design and fifteen hybrid combinations were obtained. Hybrid combinations and their parental lines were used in our study. The objective of the study was to examine genetic polymorphism of parental lines, as well as to determine relationships between SSR genetic distance and values of high parent heterosis and specific combining abilities for grain yield. By using 19 SSR primers we classified inbred lines in two groups. First cluster consists of inbred lines L1, L2, L3, L4 and L5, while inbred line L6 formed second cluster, indicating different heterotic background in comparison to the other five inbred lines. The Spearman's correlation coefficients between SSR based genetic distance and specific combining abilities for grain yield had a value of (r=0,53*), while positive correlation were also found between genetic distance and high parent heterosis (r=0,57*).

*Key words:* inbred lines, SSR genetic distance, Spearman's correlation coefficients

## INTRODUCTION

Prediction of hybrid performance is one of the main goals in almost all maize hybrid breeding programmes. Information on germplasm diversity and relationships among elite materials is of great importance in maize hybrid development (HALLAUER and MIRANDA, 1988; CHOUKAN and WARBURTON, 2005, RISTIĆ *et al.*, 2013, VANČETOVIĆ *et al.*, 2015.). Maize breeders extensively exploited phenomenon of heterosis although its genetic basis is still not completely understood. It is well known that the best hybrid combinations are obtained by crossing parental

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lines of distant genetic background (BUHINIČEK *et al*., 2009). However, MOLL *et al*., (1965) reported that increase of genetic distance between parental lines, values of heterosis increase up to the certain level, after which decline. Better understanding of genetic diversity is useful in planning crosses for hybrid and line development, in assigning lines to heterotic groups and in plant variety protection (PEJIC *et al*., 1998). Before the introduction of interdisciplinary approach in breeding programs, assessment of genetic background was valued with usage of morphological markers and later testcrosses that followed. Such approach demands a lot of time and labor work which increase costs and postpones the most important goal of maize breeding programs, which is creating promising and high yielding hybrids. On the contrary, development of laboratory techniques which allow us the insight in sole essence of divergency of breeding material, which are molecular markers, provides breeders possibility of speeding things up with greater accuracy. SSR molecular markers (microsatellites) are short segments of DNA sequences which usually consist of one to five nucleotides, which are repeatable (BRUFORD and WAYNE, 1993). Microsatellites are characterized by a high level of polymorphism, codominant effect and reproducibility, and have found application in genome mapping, identification of genotypes and population surveys.

The relationship between DNA marker based genetic distance and single cross maize hybrid yields were studied by many researchers (BETRAN *et al*., 2003; REIF *et al*., 2003; XIU *et al*., 2004; PHUMINCHAI *et al*., 2008; MLADENOVIĆ DRINIĆ *et al*.,2012). The objective of the study was to examine genetic polymorphism between parental lines, as well as to determine relationships between SSR genetic distance and values of high parent heterosis and specific combining abilities for grain yield.

## MATERIALS AND METHODS

Six maize inbred lines for which we assumed that have a similar genetic origin were chosen for this study. Selected inbred lines were crossed according to incomplete diallel design (Griffing model II), obtaining 15 single-cross hybrids. Inbred lines and single-cross hybrids obtained from them were examined in two separate trials in 2010 using RCB experimental design. Maize genotypes were tested at two locations in two repetitions. Sowing was done mechanically and during the stages of seven leaves thinning was performed. Each plot consisted of two rows, 5 m long with interrow distance of 75 cm. Harvest was done manually.

In order to determine genetic distance between examined inbred lines, 19 SSR primers were used. Isolation of DNA was done according to the modified protocol to SAGHAI and MAROOF (1984). Genetic distance between inbred lines was determined using Simple matching coefficient (SM). Sequences for these primers and their position on chromosomes are presented in Table 1.

The similarity matrix was submitted for hierarchical cluster analyses of unweighted pair group using arithmetical average (UPGMA) method and necessary computation were performed using NTSYS-pc program (ROHLF, 2000).

Analysis of specific combining ability was done using Griffing (1956), method 2, model 1. Heterosis was estimated as a high-parent (HP) heterosis. High-parent heterosis (HPH) was calculated as HPH= [(F1-HP)/HP] x 100; where, F1 is the mean of the F1 hybrid performance and HP is the mean of the best parent, where, F1 is the mean of the F1 hybrid performance and HP is the mean of the best parent.

*Table 1. SSR markers, bin numbers and primer sequences*

| SSR loci | Bin | Primer sequences |
|---|---|---|
| umc 2235 | 1.1 | 5’-TCGTCCCAGTACCATGCCTC-3’<br>5’-GAACCCTCTAGGCTCCGGTTC-3’ |
| umc 2248 | 2 | 5’-GGAACCCATCTCGCTACTAGCTC-3’<br>5’-CTCCGGTTTAATTTCTCCTCGAC-3’ |
| bnlg 198 | 2.1 | 5’ –GTTTGGTCTTGCTGAAAAATAAAA-3’<br>5’-GCTGGAGGCCTACATTATTATCTC-3’ |
| bnlg 1350 | 3.1 | 5’-TGCTTCAGCGCATTAAACTG-3’<br>5’-TGCTCGTGTGAGTTCCTACG-3’ |
| umc 1288 | 4 | 5’-ATCCGGACAAATTGAACTTTCATC-3’<br>5’-ATAGATTCAGTGTTGGACCGAGGA-3’ |
| bnlg 557 | 5 | 5’-CGAAGAAACAGCAGGAGATGAC-3’<br>5’-TCACGGGCGTAGAGAGAGA-3’ |
| phi 085 | 5.1 | 5’-AGCAGAACGGCAAGGGCTACT-3’<br>5’-TTTGGCACACCACGACGA-3’ |
| phi 126 | 6 | 5’-TCCTGCTTATTGCTTTCGTCAT-3’<br>5’-GAGCTTGCATATTTCTTGTGGACA-3’ |
| umc 1006 | 6 | 5’-AATCGCTTACTTGTAACCCACTTG-3’<br>5’-AGTTTCCGAGCTGCTTTCTCT-3’ |
| bnlg 1443 | 6.1 | 5’-TACCGGAATCCTCTTTGGTG-3’<br>5’-TTTGACAACCTCTTCCAGGG-3’ |
| umc 1859 | 6.1 | 5’-ATATACATGTGAGCTGGTTGCCCT-3’<br>5’-GCATGCTATTACCAATCTCCAGGT-3’ |
| umc 1393 | 7 | 5’-CCTTCTTCTTATTGTCACCGAACG-3’<br>5’-GCCGATGAGATCTTTAACAACCTG-3’ |
| umc 1426 | 7 | 5’-TAGGGTCGATTCTGGATTGTCTG-3’<br>5’-TGTAAAACAGAAAGCATGCGAGTC-3’ |
| umc 1695 | 7 | 5’-CAGGTAATAACGACGCAGCAGAA-3’<br>5’-GTCCTAGGTTACATGCGTTGCTCT-3’ |
| umc 1414 | 8 | 5’-GTTGACGACGTCTGGCTCCT-3’<br>5’-CGATCATCTCTCACTCTCTCGTCA-3’ |
| umc 1040 | 9 | 5’-CATTCACTCTCTTGCCAACTTGA-3’<br>5’-AGTAAGAGTGGGATATTCTGGGAGTT-3’ |
| bnlg 1506 | 9.1 | 5’-AAAGCCTCAGAGCTTCAACG-3’<br>5’-GCAGGCAACAACCAACAATA-3’ |
| umc 1507 | 10 | 5’-GATTCAAACCAAACACTTTTCCCA-3’<br>5’-CGAACCTTGCTGTGTGTTTATCAG-3’ |
| umc 1827 | 10 | 5’-GCAAGTCAGGGAGTCCAAGAGAG-3’<br>5’-CCACCTCACAGGTGTTCTACGAC-3’ |

Specific combining abilities (SCA) for crosses were calculated using following formula:

$$Sij = Xij\frac{1}{p+2}\left[\left(Ti+ii\right)+\left(Tj+jj\right)\right]+\frac{2}{\left(p+1\right)\left(p+2\right)}GT$$

Tj + jj = total j- row + average value of parent j.

The correlation between values of SCA and GD, as well as between HPH and GD for grain yield were calculated using Spearman's rank correlation coefficient HADŽIVUKOVIĆ (1971).

## RESULTS

Nineteen SSR primers which we used in our study produced 90 polymorphic bands with an average value of 4.74 per primer. Number of alleles per primer ranged from 1 to 9. Genetic distances (GD) among pairs of inbred lines ranged from 0.08 between inbred lines L2 and L3 to 0.65 between inbred lines L2 and L6, with an average value of 0.36 (Table 2).

*Table 2. Genetic distances among examined inbred lines*

| Genotype | L1 | L2 | L3 | L4 | L5 |
|---|---|---|---|---|---|
| L2 | 0,15 | | | | |
| L3 | 0,20 | 0,08 | | | |
| L4 | 0,36 | 0,30 | 0,27 | | |
| L5 | 0,30 | 0,29 | 0,30 | 0,39 | |
| L6 | 0,61 | 0.65 | 0,61 | 0,64 | 0,58 |

Figure 1 presents the dendrogram for inbred lines, according to the UPGMA clustering method. Six inbred lines, examined in our study were separated into two distinct groups. First cluster consists of inbred lines L1, L2, L3, L4 and L5, while inbred line L6 formed second cluster, indicating different heterotic origin compared to the other five inbred lines. Furthermore, within the first cluster inbred lines L2, L3 and L1 are strongly linked one to each other, while inbred lines L4 and L5 are loosely linked to inbred lines L2, L3 and L1.

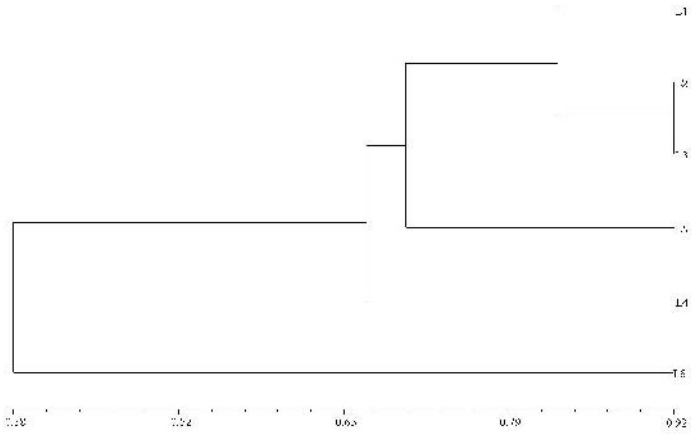

Figure 1.Dendrogram of examined inbred lines

Grain yields of parental inbred lines and their crosses as well as values of high parent heterosis and specific combining ability for grain yield are presented in Table 3.

*Table 3. Grain yields (t/ha), values of specific combining abilities and % of high parent heterosis of examined genotypes*

| Genotype | GY | SCA | %HPH | Genotype | GY |
|---|---|---|---|---|---|
| L1xL2 | 5.31 | -0.37 | 41,52* | L1 | 3.75 |
| L1xL3 | 4.83 | -0.65 | 28.58 | L2 | 3.20 |
| L1xL4 | 9.15 | 2,13** | 129,37** | L3 | 3.21 |
| L1xL5 | 9.35 | 2,33** | 166,51** | L4 | 4.09 |
| L1xL6 | 7.66 | 1,27* | 104,02** | L5 | 3.52 |
| L2xL3 | 5.08 | 0.02 | 58,32** | L6 | 2.89 |
| L2xL4 | 8.29 | 1,03* | 99,48** | | |
| L2xL5 | 7.86 | 1,23* | 123,24** | | |
| L2xL6 | 7.20 | 2,20** | 155,51** | | |
| L3xL4 | 8.29 | 1,62** | 101,56** | | |
| L3xL5 | 7.49 | 1.09 | 113,21** | | |
| L3xL6 | 6.97 | 1,21* | 117,26** | | |
| L4xL5 | 10.78 | 2,60** | 163,51** | | |
| L4xL6 | 9.04 | 1,24* | 114,63** | | |
| L5xL6 | 8.52 | 1,66** | 155,44** | | |

GY- grain yield (t/ha), SCA-specific combining ability, HPH- high parent heterosis

Parental lines yielded from 2.892 t/ha (inbred line L6) to 4.093 t/ha (L4), with an overall mean of 3.444 t/ha. On the other side, yield of crosses were in range from 4.827 t/ha (L1xL3) to 10.780 t/ha (L4xL5), with an average value of 7.721 t/ha. Values of specific combining abilities for hybrid combinations were in range from -0.65 for combination L1xL3 to 2.60** for L4xL5. For two crosses negative values of SCA were obtained, while for eleven crosses statistically significant values were observed. When it comes to the values of high parent heterosis, those were in range from 28.58% (L1xL3) to 166.51%** (L1xL5). The Spearman's correlation coefficients between SSR based genetic distance and specific combining abilities for grain yield had a value of r=0.53*, while positive correlation was also observed between genetic distance and high parent heterosis (r=0.57*).

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# MEĐUZAVISNOST GENETIČKE DISTANCE, POSEBNIH KOMBINACIONIH SPOSOBNOSTI I HETEROZISA KOD KUKURUZA (*Zea mays* L.)

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Izvod

U radu je ispitivano šest samooplodnih linija kukuruza za koje smo pretpostavljali da imaju sličnu genetsku osnovu. Samooplodne linije ukrštene su po modelu nepotpunog dialela pri čemu je dobijeno petnaest hibridnih kombinacija. U radu su ispitivane hibridne kombinacije i njihove roditeljske komponente. Cilj rada je bio da se ispita genetička divergentnost roditeljskih komponenti, kao i da se utvrdi međuzavisnost između genetičke distance izračunate korišćenjem SSR markera, vrednosti heterozisa u odnosu na boljeg roditelja i posebnih kombinacionih sposobnosti za prinos zrna. Korišćenjem 19 SSR prajmera samooplodne linije kukuruza podeljene su u dve grupe. Prvu grupu čine samooplodne linije L1, L2, L3, L4 i L5, dok se u drugoj grupi nalazi linija L6, što ukazuje na njeno drugačije heterotično poreklo u odnosu na preostalih pet samooplodnih linija. Sperman-ov korelacioni koeficijent između vrednosti genetske udaljenosti izračunate pomoću SSR markera i posebnih kombinacionih sposobnosti za prinos zrna imao je vrednost (r=0,53*), a pozitivan korelacioni koeficijent je takođe utvrđen između genetičke distance i vrednosti heterozisa u odnosu na boljeg roditelja (r=0,57*).

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